also be involved in the physiological and pathological process of articular cartilage.
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349
PHASE I CLINICAL TRIAL OF INTRA-ARTICULAR INJECTION OF AUTOLOGOUS MESENCHYMAL STEM CELLS FOR THE TREATMENT OF WRIST CHONDRAL DEFECT

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Introduction: Wrist chondral defect is a common cause of persistent joint pain, which may lead to functional impairments including reduced range of motion and diminished grip strength, affecting working ability and quality of life. There are various reported surgical treatment regimens but their effectiveness remains controversial due to the inherently poor regeneration ability of cartilage. Bone marrow-derived mesenchymal stem cells (MSC) are reported extensively to promote the regeneration of articular cartilage in various chondral defect and osteoarthritis animal models. While translation studies are on-going in knee OA, it is of clinical interest to explore the potential effectiveness of MSC therapy for the treatment of wrist chondral defect. As the first local MSC trial for chondral defect, we aimed to examine the feasibility and the safety, and to obtain data for sample size estimation for future study.
Subjects and methods: In this phase I single-arm trial, 10 patients (18 to 75 years old) with persistent post-traumatic chronic wrist pain, with imaging and previous arthroscopic evidence of wrist carpal bone chondral defects who opted for active treatment were invited to join (CREC Ref No. 2014.291-T). After arthroscopic washout and debridement, 10mL bone marrow was aspirated and subjected to the isolation and expansion of MSC cells in a certified clean room (ISO class 7). The MSC was characterized with a colony forming unit (CFU) assay, surface phenotypes (CD45, CD14, CD19, CD34, CD73, CD105, CD44, CD29, CD90, and HLA-DR), and a multipotent differentiation assay according to the International Society for Cellular Therapy guidelines. One month after bone marrow aspiration, characterised autologous MSCs (1 million cell per mL saline) with viability over 90% and clean from microbiological tests were injected back into the wrist joint of the patient. One-year follow-up assessments including functional wrist performance score and pain score, and other secondary assessments were carried out by trained personnel. The spread of data was tested for normality. Changes from baseline (pre-op) in all the measurements were determined with a t-test or Wilcoxon sign rank test where appropriate. Missing data was replaced with imputation under a missing-at-random assumption. Differences were considered statistically significant when $p < 0.05$ (SPSS V19).
Results: Six patients (5 male and 1 female), mean age of 38.5 years old fulfilling the inclusion and exclusion criteria were recruited during the reported period (January to December 2015). All procedures, including bone marrow aspiration, arthroscopic debridement, and intra-articular MSC injection, were uneventful and there were no signs of infection and nil complications noted or reported. It was practical to expand MSCs *in vitro* to a sufficient number for characterisation and injection in one month. Till now, six-month follow up data indicated the potential therapeutic effect of intra-articular MSC injection at single dose, as shown by numerical improvement in performance score and pain score.
Discussion and conclusion: This pilot clinical trial shows the safety and potential therapeutic effect of single dose autologous bone marrow-derived MSCs on persistent wrist chondral defect. Additional data from the second phase follow-up will provide more insight into the treatment of wrist chondral defect with MSCs.
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358
OPTIMISATION OF CULTURE CONDITIONS FOR MAINTAINING PORCINE INDUCED PLURIPOTENT STEM CELLS

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Ground state porcine induced pluripotent stem cells (piPSCs), which retain the potential to generate chimeric animal and germline transmission, are difficult to produce. This study investigated morphological and biological progression at the early stage of porcine somatic cell reprogramming and explored suitable conditions to increase the induction efficiency of piPSCs. A cocktail of defined transcription factors was used to generate piPSCs. The amphotropic retrovirus, which carried human OCT4 (O), SOX2 (S), KLF4 (K), C-MYC (M), TERT (T), and GFP were used to infect porcine embryonic fibroblasts (PEFs). The number of clones derived from OSKM (4F) and OSKMT (4F + T) was significantly higher than that from SKM (3F) and SKMT (3F + T), suggesting that OCT4 played a critical role in regulating porcine cell reprogramming. The number of alkaline phosphatase positive clones from a medium with leukaemia inhibitory factor (LIF) and basic fibroblast growth factor (bFGF) (M1 medium) was significantly higher than that with insulin and 2i PD0325901/CHIR99021 (M2 medium), indicating that insulin and 2i could not effectively maintain piPSC propagation. In the M1 medium, piPSC lines could not maintain the typical self-renewal morphology on gelatin-coated and Matrigel-coated plates. Without the mouse embryonic fibroblast (MEF) feeder, piPSCs started to simultaneously differentiate. Based on the potential for self-renewal and activation of pluripotent markers, we found that the culture condition of 4F + T plus LIF and bFGF plus MEF feeder promoted PEF reprogramming more efficiently than the other conditions tested. Two piPSC lines (IB-1 and IB-2) were derived and maintained for up to 20 passages *in vitro*.
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405
INJECTABLE AND ROBUST BIOPOLYMER-BASED SUPRAMOLECULAR HYDROGELS FOR REGENERATIVE MEDICINE

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Osteoarthritis (OA), which is symptomised as progressive degradation of articular cartilage in human diarthrodial joints, has become one of most prevalent, debilitating diseases in modern society. To address the increasing clinical demand for more effective treatment of OA, significant progress has been made in biotechnology, especially in the field of biomaterials. In the most recent decade, increasing research emphasis has been placed on the "bio" part of biomaterials. In our lab, we have shown that functionalisation of the hydrogels with biomimetic peptides promotes the differentiation of the hMSCs. In addition to the biofunctionalisation, the physical functions of the biomaterials are also critical to the successful translation of biomaterials to clinical treatment of cartilage diseases. Although biopolymer-based chemical hydrogels, with biopolymers covalently crosslinked, have been widely used as scaffolds for tissue engineering due to good stability, their permanent network structures and brittleness limit their applications in repairing load-bearing tissues, such as cartilage. In contrast, biopolymer-based supramolecular hydrogels, which are usually formed via self-assembly of physically interacting biopolymers are usually weak, as shown in "inverted vials", instead of freestanding 3D constructs and they are less stable than chemical hydrogels. Herein, we describe a novel host-guest macromer (HGM) approach for preparation of biopolymer-based freestanding supramolecular hydrogels. We have developed a series of injectable hydrogels with unique properties such as resilient mechanical property, bioadhesiveness, injectability, and promoting recruitment of endogenous cells that are desirable for potential clinical applications in the regeneration of soft musculoskeletal tissues such as cartilage, meniscus, and intervertebral discs.
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410
CO-CULTURE OF HUMAN SYNOVIUM-DERIVED STEM CELLS AND CHONDROCYTES REDUCES HYPERTROPHY

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Introduction: Mesenchymal stem cells (MSCs) have emerged as a clinically relevant cell source for regenerative medicine, especially for cartilage repair; however, it still remains a challenge to recapitulate the functional properties of native articular cartilage using only MSCs. *In vitro* expansion of chondrocytes causes dedifferentiation. Lately, co-cultures of chondrocytes and bone marrow MSCs demonstrated enhanced functional properties of engineered cartilage. In this study, we aimed to assess the effect of co-culture of synovium-derived stem cells (SDSCs) and chondrocytes on *in vitro* chondrogenesis in serum-free TGF-β supplemented medium.
Methods: Isolation and expansion of cells: Human SDSCs and chondrocytes were isolated by sequential digestion from explants of total knee arthroplasty patients and incubated at 37 °C, 95 % humidity, and 5 % CO_2 in standard culture medium. We used passage 2 cells hereafter.
In vitro differentiation of cells: Expanded chondrocytes, SDSCs, and chondrocyte/SDSCs (5×10^5cells per pellet; co-culture ratio, 1:1) were cultured in chondrogenic medium for 1, 7, 14, and 21 days. The SDSC and chondrocyte/SDSC mixed pellets were cultured with the supplementation of 10 ng/mL of TGF-β1 and the chondrocyte pellets were cultured in chondrogenic medium only. The medium was changed twice per week.